

PCT/CA 2005/000250

- 71 -

06 DECEMBER 2005 06-12.05

CLAIMS

1. A nucleic acid sequence according to any one of the sequences in Table 1 for use in a PCR primer pair for multiplex SNP analysis of a plurality of blood group- or platelet antigen SNPs simultaneously.
2. A set of oligonucleotides comprising at least one primer set of Table 1, wherein said set of oligonucleotides is suitable for amplifying and detecting a plurality of blood group or HPA SNPs simultaneously in a single tube.
3. A nucleic acid sequence according to any one of the sequences in Table 2.
4. A nucleic acid sequence according to claim 3 for use as extension probes for the identification of SNPs.
5. A nucleic acid sequence according to claim 1 or 4, wherein said SNPs relate to blood group and platelet antigens.
6. An oligonucleotide set according to claim 2, wherein said at least one oligonucleotide hybridizes a HPA-1 GP3A SNP for the determination of the HPA genotype and corresponding phenotype.
7. An oligonucleotide primer and probe set for analyzing a plurality of SNPs corresponding to a blood group or platelet antigen genotype, simultaneously; wherein said plurality of SNPs are selected from the group consisting of RhD RHD Exon 4 C/T; RhD RHD Exon 9 A/G; RhC/c RHCE Exon 2 T/C; RhE/e RHCE Exon 5 C/G; S/s GYPB Exon 4 T/C; K/k KEL

PCT/CA 2005/000250

- 72 -

06 DECEMBER 2005 06-12.05

Exon 6 T/C; Kp^a/Kp^b KEL Exon 8 T/C; FY/FY⁰ FY
Promoter T/C; Fy^a/Fy^b FY Exon 2 G/A; Jk^a/Jk^b KIDDExon
9 G/A; Di^a/Di^b DIEGO Exon 19 T/C; and HPA-1a/b GP3A
Exon 3 T/C.

8. An oligonucleotide primer and probe set for analyzing the SNPs of claim 7, wherein one, more than one or all of said primer set is selected from Table 1, and wherein one, more than one of all of said probe set is selected from Table 2, such that the selection of primer and probe combinations correspond to the SNP being analyzed.
9. A method of simultaneously analyzing a plurality of blood group or platelet antigens in a sample wherein said method comprises:
 - (a) isolation and purification of genomic DNA from said sample;
 - (b) multiplex PCR amplification of DNA regions encompassing a plurality of SNPs of interest, each corresponding to a blood group or platelet antigen genotype;
 - (c) digestion of multiplex PCR amplified products with restriction enzymes;
 - (d) identification of SNPs using single-base pair primer extension of the amplified DNA fragments using the probes of Table 2;
 - (e) hybridization of extension products; and
 - (f) analysis of SNP extension products to determine a genotype corresponding thereto.

10. A method according to claim 9, wherein said restriction enzymes are Exonuclease I and Shrimp alkaline phosphatase for the purpose of removing excess dNTPs and/or oligonucleotides.
11. A method according to claim 9, wherein said extension products are hybridized to tag-arrayed microplate.
12. A method according to claim 9, wherein the multiplex PCR amplification comprises amplification with nucleotides primer and probes selected from Tables 1 and 2.
13. A method according to claim 9, wherein a thermal cycler is used to carry out the single-pair primer extension.
14. A method according to claim 9, wherein any machine or method capable of analyzing SNPs may be used.
15. A method according to claim 9, wherein GenomeLab SNPstream (Beckman Coulter Inc.) is used to analyze SNP extension products.
16. A method of claim 9, wherein said method is carried out in a single reaction tube or single well of a multiwell plate.
17. A method of claim 9, wherein said method is automated.
18. A method according to claim 9, wherein said antigens are red blood cell and platelet blood group antigens.
19. A method according to claim 9, wherein said antigens are selected from the group consisting of

PCT/CA 2005/000250
06 DECEMBER 2005 06-12.05

- 74 -

ABO, Rh (D, C, c, E, e), MNS, P, Lutheran, Kell (K, k), Lewis, Duffy (Fy^a, Fy^b), Kidd (Jk^a, Jk^b).

20. A method for the simultaneous detection of the presence or absence of blood cell antigen SNPs simultaneously using one or more of the the oligonucleotides of Table 1 and Table 2, or any corresponding combination thereof.
21. A method according to any one of claims 9 to 20, wherein 12 blood group and HPA SNPs are analyzed in a single tube.
22. A method according to claim 9, wherein said SNPs identified in step (d) include a HPA-1 GP3A SNP which is analyzed for the determination of HPA genotype and corresponding phenotype.
23. A multiplex PCR method for the identification of blood group genotypes, comprising identifying and analyzing the corresponding SNPs combinations thereof according to the following steps:
 - (a) isolation and purification of genomic DNA from said sample;
 - (b) multiplex PCR amplification of DNA regions encompassing said plurality of blood group SNPs, and including a plurality of primer pairs of Table 1;
 - (c) the digestion of multiplex PCR amplified products with restriction enzymes;
 - (d) identification of SNPs using single-base pair primer extension of the amplified DNA fragments;
 - (e) hybridization of extension products; and

PCT/CA 2005/000250

- 75 -

06 DECEMBER 2005 06-12.05

(f) analysis of SNP extension products to determine the SNP genotype; wherein said analysis simultaneously screens a plurality of SNPs in a single reaction tube.

24. A method according to claim 14, wherein said test sample is a human blood sample.
25. A method according to claim 14, wherein said plurality of SNPs are selected from the group consisting of RhD RHD Exon 4 C/T; RhD RHD Exon 9 A/G; RhC/c RHCE Exon 2 T/C; RhE/e RHCE Exon 5 C/G; S/s GYPB Exon 4 T/C; K/k KEL Exon 6 T/C; Kp^a/Kp^b KEL Exon 8 T/C; FY/FY0 FY Promoter T/C; Fy^a/Fy^b FY Exon 2 G/A; Jk^a/Jk^b KIDDExon 9 G/A; Di^a/Di^b DIEGO Exon 19 T/C; and HPA-1a/b GP3A Exon 3 T/C.
26. The use of nucleic acid sequences of Tables 1 and 2 in multiplex PCR for the identification and analysis of blood group or platelet antigen SNPs.
27. The use according to claim 26, wherein said multiplex PCR is carried out in a single reaction tube.
28. The use according to claim 26, wherein said multiplex PCR is automated to simultaneously analyse blood group and platelet antigen SNPs.
29. The use according to claim 26, wherein said SNP analysis results in antigen genotypes and corresponding phenotypes of a test sample.
30. A method of claim 23 wherein blood group antigen and platelet antigen typing is determined using the primer pairs of Table 1, and analysis using the probes of Table 2.

PCT/CA 2005/000250**06 DECEMBER 2005 06-12.05**

31. A method of claim 30, wherein said typing uses a multiplex PCR SNP analysis format, wherein said analysis is automated.
32. A method of simultaneously analyzing a plurality of blood group or platelet antigens in a sample wherein said method comprises:
- (a) isolation and purification of genomic DNA from said sample;
 - (b) multiplex PCR amplification of DNA regions encompassing a plurality of SNPs of interest, using a plurality of primer pairs of Table 1;
 - (c) digestion of multiplex PCR amplified products with restriction enzymes;
 - (d) identification of SNPs using single-base pair primer extension of the amplified DNA fragments using probes corresponding to said SNPs of interest;
 - (e) hybridization of extension products; and
 - (f) analysis of SNP extension products to determine the SNP genotype.
33. The method of claim 23, wherein said step of identification of SNPs includes using the probes of Table 2.
34. The method of claim 23, wherein said step of hybridization includes using the probes of Table 2.
35. The method of claim 23, wherein said blood group SNPs includes a SNP of Table 1 or Table 1A.
36. The method of claim 30 wherein said blood group antigen and platelet antigen are human antigens.